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FILTRATION CHARACTERISTICS OF MS2 BACTERIOPHAGE USING VARIOUS MOLECULAR WEIGHT FILTERS

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MS2 Bacteriophage has a reported nominal molecular weight of 2M Dalton. It would be expected that this phage would not pass through filters of various sizes with low molecular weight cut off (MWCO) values of less than IM Dalton. It was discovered that MS2 Bacteriophage will pass through filters with 750 K Dalton, 500 K Dalton, and 300 K Dalton MWCO values. MS2 was retained on the 100K Dalton filter. A cross flow hollow fiber apparatus was used for the 750 and 500 K Dalton analysis. Centrifuge filters of 1M and 300K and 100K Dalton were used. The rate of passage of MS2 through the cross flow filters is dependent upon the tangental flow rate and pressure. Passage through the centrifuge filters depended upon the gravitational force applied.

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Preface

The work described in this report was performed as part of a Defense Advanced Research Projects Agency (DARPA), Defense Sciences Office, project. This work was started in March 1998 and was completed in September 1998.

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Filtration Characteristics of MS2 Bacteriophage Using Various Molecular Weight Filters

1. Introduction

Nominal molecular weight cut off values (MWCO) of various filters can lead to the assumption that items larger than the cut off values will be retained after filtration. It was discovered that, at least for MS2 bacteriophage, there are exceptions. It was discovered during the operation of the Integrated Virus Detection System (IVDS) instrument that counts of MS2 decreased during ultrafiltration and purification. This was an important discovery in that for the detection of small numbers of viruses any loss is important. This observation lead to further investigation.

As a result, this study was initiated to better understand the filtration characteristics of MS2 bacteriophage. The ability of differing filtration techniques and their relative filtration effectiveness was explored. The sample of MS2 bacteriophage, used in the filtration studies, was received from the Life Sciences Division at Dugway Proving Ground (DPG). This sample was 2 ml of purified MS2 bacteriophage at a concentration $1x10^{14}$ pfu/ml or 10.2 mg protein/ml. This highly purified sample is from Lot #98110.

The two types of filters used in this study were a centrifuge tube assembly, where the solution is forced through the filter by gravitational forces and a cross flow filter apparatus with pressure pushing the solution through the filter. The centrifuge filter assemblies are available in various sizes and molecular weight cut off (MWCO) filter inserts. The MWCO is changed to capture biological material, such as proteins, cell products and viruses, by molecular weight differentiation. The cross flow filter, or ultrafiltration apparatus, is also used to capture or reject biological material by adjusting the MWCO of the filter. These filtration systems operate by pumping the feed stream through a hollow fiber. As the solution passes through the fiber, the sweeping action of the flow helps to prevent clogging of the fiber. A pressure differential forces the filtrate through the fiber, while the biological feed stream is purified and concentrated. There are available a wide range of pore sizes for the centrifuge filters as well as the hollow fiber filters.

The MS2 samples were analyzed after filtration using the IVDS instrument or more directly the Gas-phase Electrophoretic Mobility Molecular Analyzer (GEMMA) detector which is one stage of the IVDS instrument. The GEMMA detector consists of an electrospray unit to inject samples into the detector, a Differential Mobility Analyzer and a Condensate Particle Counter. A complete description of the IVDS system, including the GEMMA detector, can be found in the report *Virus Detection: Limits and Strategies.*[1]

[1] Wick, C.H., Yeh, H.R., Carlon, H.R., and Anderson, D., *Virus Detection: Limits and Strategies*, ERDEC-TR-453, December 1997.

2. Laboratory Testing

2.1 Results

The first set of solutions produced consist of $1x10^{11}$ pfu/ml of MS2 in a cesium chloride (CsCl) solution (0.5%, by weight) in an ammonium acetate buffer (0.02M). The procedure in these cases was to place 150 µl of the solution into a wedge filter of differing molecular weight cut-off (MWCO). The MWCO used were 30K, 50K and 100K Dalton. The filter was then centrifuged and the samples were analyzed in the GEMMA. As shown in Table 1, the wedge filters all concentrated the MS2 solution, i.e. the counts increased as the solution size decreased. Even with a subsequent addition of buffer and re-centrifugation, the solutions continue to concentrate.

The same solution (CsCl 0.5% + $1x10^{11}$ pfu/ml MS2) was then placed into a 1M Dalton centrifuge filter and spun. The first concentration shows an increase (see Table 1) from 150 counts to 350 counts in the sample. The solution volume decreasing, from 1000 to 100 µl, should increase the counts measured. The subsequent wash and re-centrifugation should show an increase in MS2 counts. However, the counts for the washed sample are even lower. The conclusion from the filtration with the 1M MWCO filter is that the MS2 bacteriophage is able to pass through the filter and is not retained.

Table 1 Filtration of MS2 plus CsCl Solutions

Sample	Filter MWCO (Daltons)	Counts	Volume (µl)	+ 1 Wash (counts)	Volume (µl)
CsCl 0.5% + $1x10^{11}$ MS2, DPG	None	150	150		
CsCl 0.5% + $1x10^{11}$ MS2, DPG	30K	2500	25	4500	35
CsCl 0.5% + $1x10^{11}$ MS2, DPG	50K	2000	20	3000	25
CsCl 0.5% + $1x10^{11}$ MS2, DPG	100K	9000	15	5000	10 (+5 buffer)
CsCl 0.5% + $1x10^{11}$ MS2, DPG	1M centrifuge	350	100	75	50

To actually determine if the MS2 is passing through the centrifuge filters would be to analyze the filtrate. A separate sample of $1x10^{12}$ pfu/ml MS2 (DPG ultrafiltration cleaned, mixed media sample) was filtered with the 1M centrifuge filters. As shown in Figure 1, the MS2 passed through the filter and was deposited in the filtrate. Table 2 presents the numerical counts from the GEMMA analysis of the retentate, after one wash cycle, and the filtrate from the 1M centrifugation of the sample.

Table 2 Filtration of MS2 Solution after Ultrafiltration Processing

Sample	Filter MWCO (Daltons)	GEMMA Counts	Volume (µl)	+ 1 Wash (counts)	Volume (µl)
DPG MS2 Mixed Media UF Mod 1	none	5000	100		
DPG MS2 Mixed Media UF Mod 1 Retentate	1M centrifuge			75	100
DPG MS2 Mixed Media UF Mod 1 Filtrate	1M centrifuge	3,500	150		

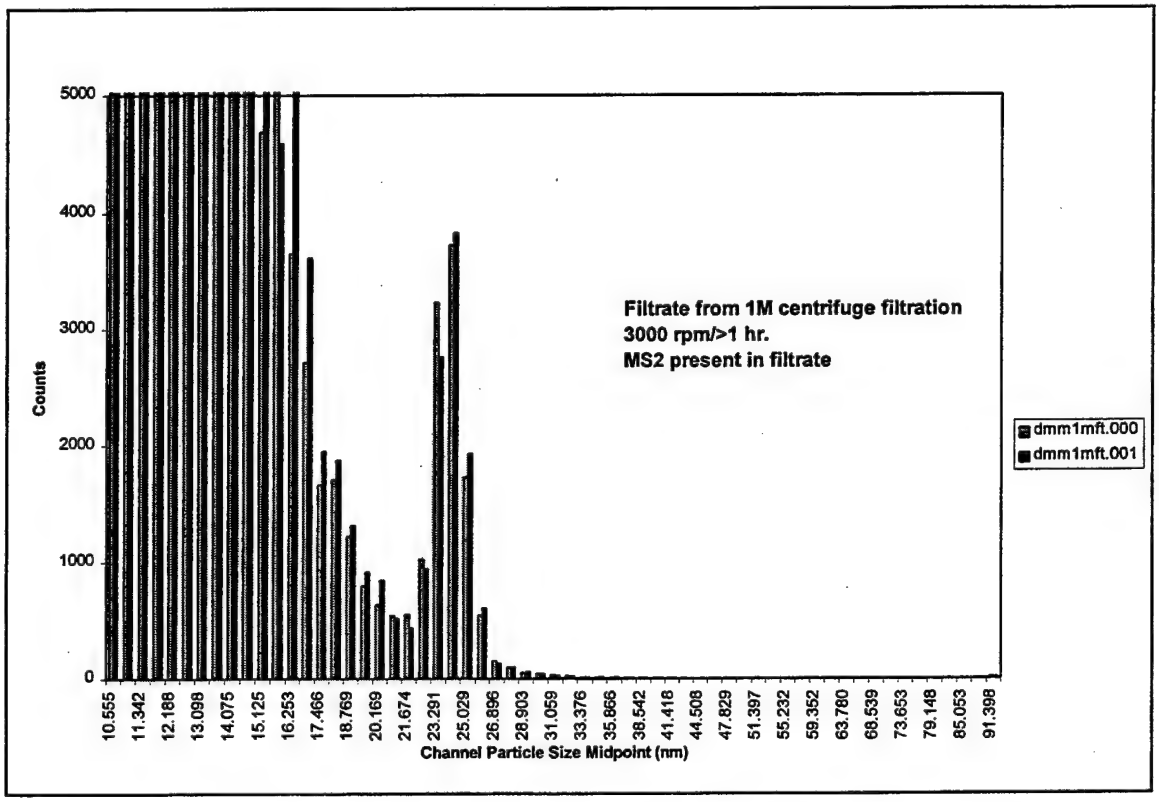

Figure 1 Filtrate from 1M (MWCO) Centrifugation of MS2 Solution

To determine if there was any interference from the CsCl during the filtration with the 1M filters, a solution of MS2 was prepared at a concentration of $1x10^{11}$ pfu/ml by dilution in the ammonium acetate buffer only. The sample was prepared from a stock solution obtained from the Life Sciences Division of Dugway Proving Ground (DPG). The MS2 solution was then centrifuged in the 1M centrifuge filter. As shown in Table 3, the plain MS2 solution also passed through the 1M filter apparatus with the loss of virus material. The CsCl does not appear to affect the loss of virus material by its presence in the filtration solution.

Table 3 Filtration of Pure MS2 Solutions

Sample	Filter MWCO (Daltons)	GEMMA Counts	Volume (µl)
$1x10^{11}$ MS2, DPG	None	600	100
$1x10^{11}$ MS2, DPG Retentate	1M centrifuge	65	100

Another type of filtration is the cross flow or tangential flow technique. The solution is pumped through a hollow fiber that is designed to allow the passage of differing MWCO materials, depending on the filter installed. A flow restriction at the exit from the fiber bundle develops a pressure differential that forces the filtrate through the fiber and concentrates the feed solution. Figure 2 is a representation of this technique.

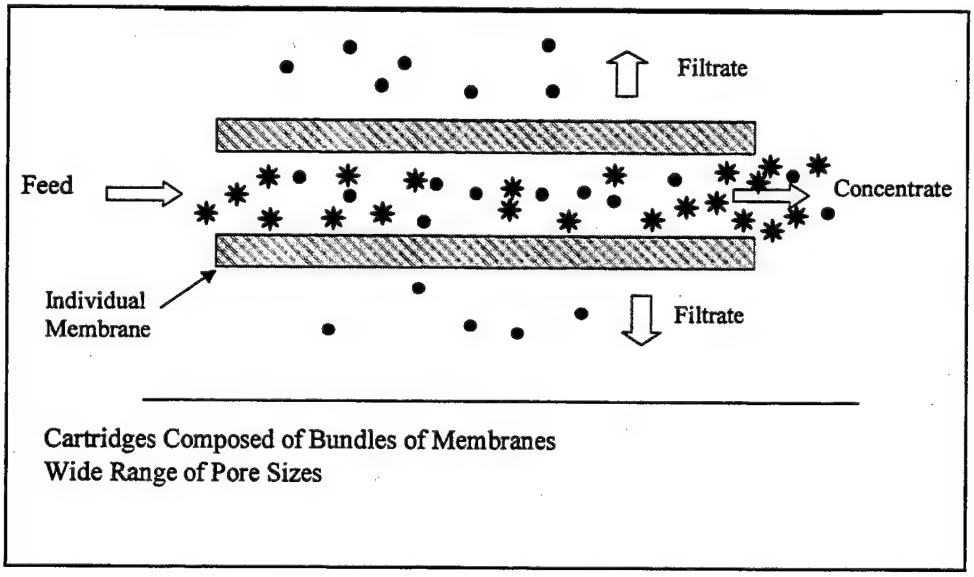

Figure 2 Cross Flow Filtration Apparatus

The first sample prepared for filtration was a CsCl (0.05%, by weight) solution with $3x10^{11}$ pfu/ml MS2 added into the ammonium acetate buffer. The ultrafiltration parameters for this solution are shown in Table 4. As shown in Table 5, the sample volume was concentrated from 1000 to 100 µl, but the counts dropped from 3200 to 25. This drop in counts shows that the cross flow filter, at a MWCO of 750K Dalton, is allowing the virus to pass through the hollow fiber.

Table 4 Cross Flow Parameters for CsCl (0.05%) plus MS2 ($3x10^{11}$ PFU)

Sample volume-initial	1 ml
Pump speed	2
Transducer pressure	15 psig
Total buffer wash volume	40 ml
Sample volume-final	0.1 ml
MWCO of module	750K

Table 5 Cross Flow Filtration of CsCl (0.05%) plus MS2 ($3x10^{11}$ PFU)

Sample	Filter MWCO (Daltons)	GEMMA Counts	Volume (µl)
CsCl 0.05% + $3x10^{11}$ MS2, DPG	None	3200	1000
CsCl 0.05% + $3x10^{11}$ MS2, DPG Retentate	UF Mod1 750K	25	100

The second sample tested, a CsCl solution (2.5%, by weight) plus $5x10^{11}$ pfu/ml MS2 in ammonium acetate buffer, was processed through the cross flow filtration apparatus with a filter of 500K MWCO. The parameters for the ultrafiltration processing of the solution are shown in Table 6. Although the sample volume was concentrated by half, the counts remained constant, as shown in Table 7. It appears that the MS2 virus is also passing through the 500K filter, although at a slower rate than the 750K filter.

Table 6 Cross Flow Parameters for CsCl (2.5%) plus MS2 ($5x10^{11}$ PFU)

Sample volume-initial	1 ml
Pump speed	2
Transducer pressure	15 psig
Total buffer wash volume	30 ml
Sample volume-final	0.5 ml
MWCO of module	500K

Table 7 Cross Flow Filtration of CsCl (2.5%) plus MS2 ($5x10^{11}$ PFU)

Sample	Filter MWCO (Daltons)	GEMMA Counts	Volume (µl)
CsCl 2.5% + $5x10^{11}$ MS2, DPG	None	800	1000
CsCl 2.5% + $5x10^{11}$ MS2, DPG Retentate	UF Mod1 500K	750	500

To test the lower limit of MWCO for a MS2 bacteriophage, a centrifuge filter of 300K MWCO was obtained. It appears from Table 1 that the filters up to 100K MWCO do not allow the passage of MS2 through the filter medium. The 300K filter was loaded with 100 µl, diluted to 1 ml in ammonium acetate buffer, of a $1x10^{11}$ pfu/ml MS2 sample from DPG. The sample was centrifuged and the retentate analyzed. As shown in Figure 3, the MS2 is at least partially retained in the 300K filter.

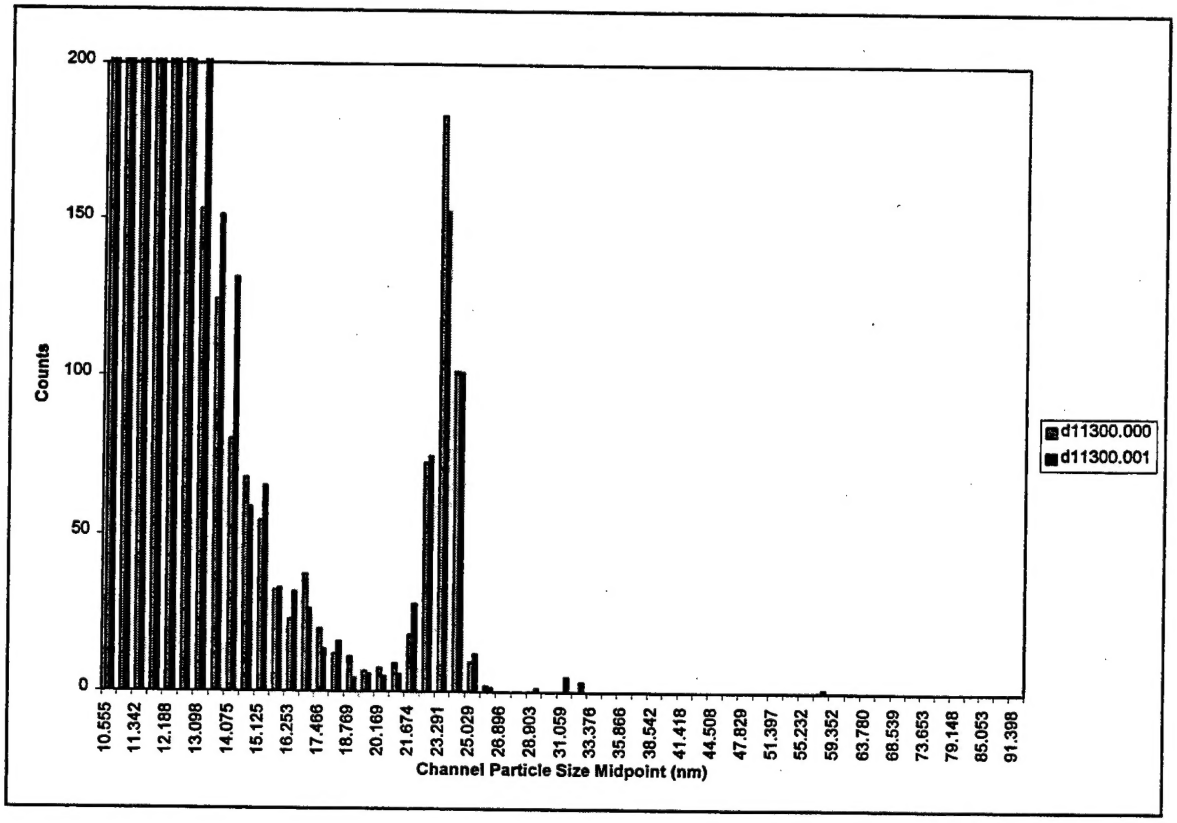

Figure 3 GEMMA Scan of $1x10^{11}$ PFU MS2 Retained on 300K MWCO Centrifuge Filter

To determine the amount, if any, of MS2 passing through the filter, a 1 ml portion of the filtrate was concentrated in the 100K wedge filters. The final volume was reduced to 25 µl. As shown in Figure 4, there was MS2 present in the filtrate from the 300K centrifuge filtration. It would appear that the MS2 is able to pass through MWCO filters as small as 300K. The MS2 does not appear to pass through the 100K centrifuge filters.

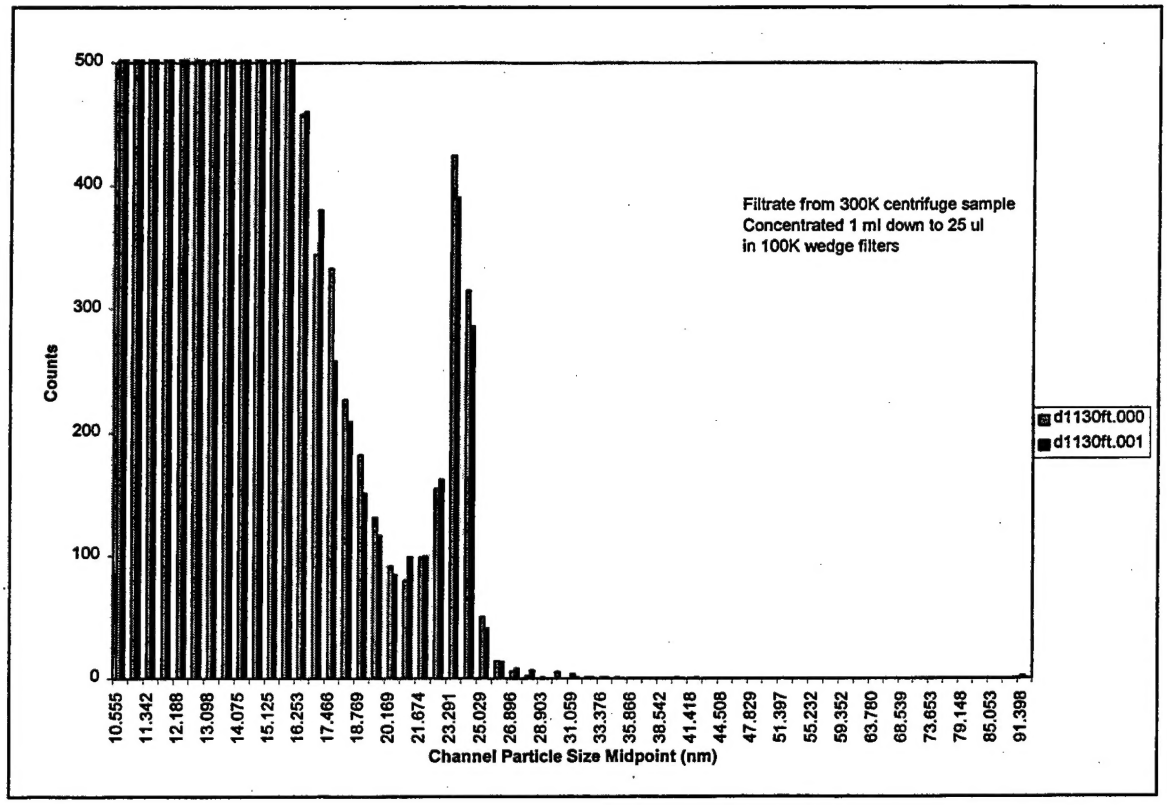

Figure 4 GEMMA Scan of $1x10^{11}$ PFU MS2 Concentrated Filtrate

A series of solutions of $1x10^{12}$ pfu/ml of MS2 bacteriophage will be filtered with the cross flow apparatus with a 750K MWCO ultrafilter installed. All of the filtered solutions will include 1 ml of the $1x10^{12}$ pfu/ml MS2 with various additions of ammonium acetate buffer solution. The additions of buffer will allow differing lengths of time of filtration, in the cross flow apparatus, while keeping the amount of MS2 in the sample constant. However, the concentration of the MS2 will vary depending on the dilution factor in the starting sample. The samples will be processed in the cross flow apparatus until concentrated to approximately the 1 ml volume of the $1x10^{12}$ pfu/ml MS2 initial sample. Table 8 presents the filtration parameters for the cross flow apparatus for this set of experiments. Table 9 shows the starting volumes, initial dilution's, final sample volume and subsequent GEMMA sample count for the MS2 viral peak.

Table 8 Cross Flow Parameters for MS2 ($1x10^{12}$ PFU) plus Variable Volume Ammonium Acetate Buffer Solutions

Sample volume-initial	1 ml MS2 + variable buffer volumes
Pump speed	2
Transducer pressure	15 psig
Total buffer wash volume	variable
Sample volume-final	0.70-0.75 ml
MWCO of module	750K

Table 9 Dilution Amounts and GEMMA Analysis of Cross Flow Filtration of MS2 Samples

MS2 Start Volume	Ammonium Acetate Dilution (ml)	Final Volume (ml)	GEMMA Counts for MS2 Peak (avg. of 2 runs)
1 ml @ $1x10^{12}$ pfu/ml	0	1.0	9255
1 ml @ $1x10^{12}$ pfu/ml	1	0.75	5164
1 ml @ $1x10^{12}$ pfu/ml	2	0.70	5280
1 ml @ $1x10^{12}$ pfu/ml	4	0.75	3239
1 ml @ $1x10^{12}$ pfu/ml	8	0.70	5284
1 ml @ $1x10^{12}$ pfu/ml	16	0.75	3549
1 ml @ $1x10^{12}$ pfu/ml	32	0.70	2830

The final volume of the solutions processed through the cross flow apparatus is essentially equivalent. The solutions should therefore exhibit the same count rate for MS2, as the initial amount of virus was equal in all cases. The count rates are plotted in Figure 5, and show a logarithmic decline as the dilutions were increased. The increased dilution's lengthened the contact time with the cross flow filter and subsequently increased the loss of the MS2 bacteriophage through the filter medium.

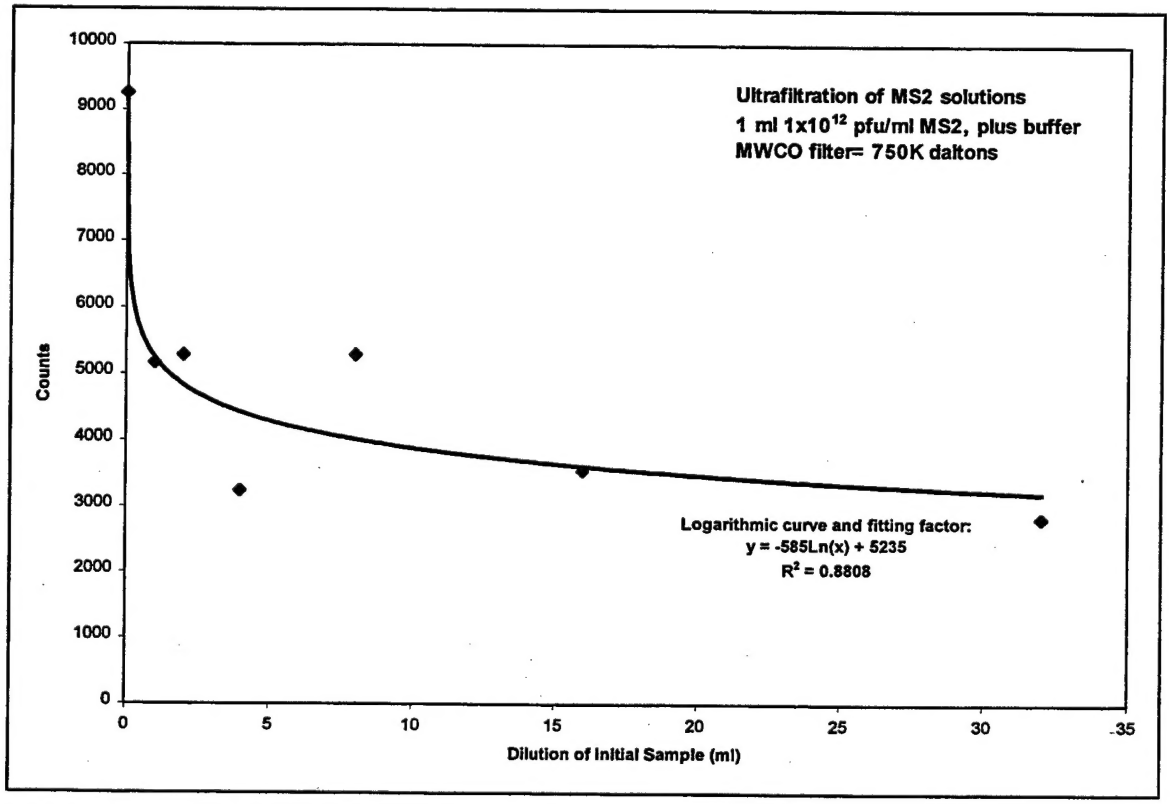

Figure 5 GEMMA Counts and Logarithmic Curve for Variable Dilution's and Cross Flow Filtration of MS2 plus Ammonium Acetate Buffer Solutions

2.2 Analysis

The MS2 bacteriophage was able to pass through the filters of MWCO of 300K and higher daltons and was retained on filters of 100K and less. This result was not expected as the bacteriophage has an approximate size of 2M daltons, and was expected to be retained on the initial filter of 750K MWCO size tested. Collins, et al observed a similar result,[2] in a report to Koch Membrane Systems, Inc. This study showed the retention of MS2 bacteriophage with MWCO filters of 100K daltons and smaller and the passage of MS2 through a 500K dalton filter. The variable dilution cross flow filtration analysis in this report shows the logarithmic removal of the MS2 from the feed stream, as the solutions were concentrated. The longer the MS2 solution was in contact with the cross flow filter of 750K, the more MS2 was removed from the solution. If the goal of cross flow filtration is to remove salts and other ionic entities, a smaller MWCO filter (such as a 100K) could be used and the MS2 would be retained. However to remove larger macromolecules from a sample of MS2 bacteriophage, a different approach would be needed. A larger MWCO filter (macromolecule dependent) would be used to retain and concentrate the macromolecule while the MS2 bacteriophage is removed in the filtrate stream. The filtrate stream could then be processed separately with a 100K MWCO filter to retain and concentrate the MS2 bacteriophage. The extra step would only add a short period of time to an analysis, as the cross flow filtration process is a fast and efficient filtration.

3. Conclusions

MS2 bacteriophage passed through various MWCO filters above 300K. MS2 seemed to be retained on the 100K MWCO (and below) filters

Variable dilutions with cross flow filtration apparatus and a 750K MWCO filter produced a logarithmic removal of the MS2 during filtration

Retention of MS2 on 300K rated hollow fibers was high enough that only minor losses would be incurred under conditions that would accomplish significant removal of even large proteins.

Implications are clear that a better understanding of molecular weight cut off (MWCO) and how pore sizes are determined and reported need to be further investigated.

This finding has important implications for investigations and procedures dependent upon retaining viruses.

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[2] Collins, M.R., Dwyer, P.L., Margolin, A.B., and Hogan, S.B., *Assessment of MS2 Bacteriophage Virus, Giardia Cyst and Cryptosporidium Oocyst Removal by Hollow Fiber Ultrafiltration (Polysulfone) Membranes*, submitted to Koch Membrane Systems, Inc. Wilmington, MA 01887